Supporting information

Striking Effect of Polymer End-group on C60 Nanoparticle Formation by High Shear Vibrational Milling with Alkyne-Functionalized Poly(2-oxazoline)s

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Materials and methods:

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler (ALS), a thermostatted column compartment (TCC) at 50°C equipped with two PLgel 5 µm mixed-D columns and a guard column in series, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). The used eluent was *N,N*-dimethylacetamide DMA containing 50 mM of LiCl at a flow rate of 0.593 mL/min. The spectra were analyzed using the Agilent Chemstation software with the GPC add-on. Molar mass and Đ values were calculated against PMMA standards from PSS. UV-Vis spectra were recorded on a Varian Cary 100 Bio UV-Vis spectrophotometer equipped with a Cary temperature and stir control. Samples were measured in either quartz or disposable cuvettes with a pathlength of 1.0 cm in the wavelength range of 200 to 700 nm. The concentration of each sample was 1.0 mg/mL in milliQ water. Matrix assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF MS) was performed on an Applied Biosystems Voyager De STR MALDI-TOF mass spectrometer equipped with 2 m linear and 3 m reflector flight tubes, and a 355 nm Blue Lion Biotech Marathon solid state laser (3.5 ns pulse). All mass spectra were obtained with an accelerating potential of 20 kV in positive ion mode and in either reflectron or linear mode. The polymerizations were performed in capped vials in a single mode microwave Biotage initiator sixty (IR temperature sensor) (Biotage, Uppsala, Sweden) following a previously reported protocol.[1] Lyophilisation was performed on a Martin Christ freeze-dryer, model Alpha 2-4 LSC plus. High Speed Vibration Milling (HSVM) was performed in a Fritsch Mini-Mill Pulverisette 23 in a 10 mL stainless steel grinding bowl with 15 mm diameter grinding ball(s). Preparative size exclusion chromatography was performed with Disposable PD-10 Desalting Columns from GE Healthcare. Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded on a Bruker Avance 300 or 400 MHz spectrometer at room temperature. The chemical shifts are given in parts per million (δ) relative to TMS. The compounds were dissolved in either $CDCl_3$, D_2O or DMSO-d6 from Eurisotop.

Unless otherwise stated, all chemicals were used as received. All HPLC grade solvents were purchased from Sigma-Aldrich (acetone, diethylether, DMA, dichloromethane, methanol, acetonitrile), from Fischer Scientific (Toluene) or from Acros (dry DMF, DMSO). All reagents were purchased from Sigma-Aldrich, with the exception of triphenyl phosphine and sodium-L-ascorbate, which were bought from Acros. γ-Cyclodextrin was kindly provided by Wacker Chemie. 2-Ethyl-2-oxazoline was kindly provided by PCI, and was further purified by distilling over BaO. Propargylbenzenesulfonate was purchased from Sigma-Aldrich and was further purified by vacuum distillation. Acetonitrille was dried through a custom-built JC Meyer solvent purification system whereby the solvents passes over an Alumina oxide column for drying. Further characterization details (NMR spectra, MALDI-TOF-MS spectra, *etc.*) on the synthesis of the described compounds are provided in the experimental section below.

Dynamic light scattering (DLS): were initially analyzed on a Zetasizer Nano-ZS Malvern apparatus (Malvern Instruments Ltd.) using disposable cuvettes. The excitation light source was a He−Ne laser at 633 nm and the intensity of the scattered light was measured at an angle of 173°. The concentration of each sample was 1.0 mg/mL in milliQ water. All samples were filtered with a 0.2 μm pore sized filter prior to measurement.

Additionally, the DLS measurements were performed using an ALV CGE laser goniometer. The scattered light of a 22 mW HeNe linear polarized laser (632.8 nm) was measured in a broad angle range 40-150^{o} , and was collected on an ALV 6010 correlator. The data were collected using the ALV correlator control software with different counting times from 100 to 300 s to accumulate an intensity correlation function $g_2(t)$ with a low signal-to noise ratio. The measured $g_2(t)$ were analyzed using the algorithm REPES (incorporated in the GENDIST program) resulting in the distributions of relaxation times – $A(\tau)$ or hydrodynamic radii W(R_h).The diffusion coefficient D was obtained from the standard relation:

$$\Gamma = \tau^{1} = D_{tr}q^{2} \qquad (1)$$

where Γ is the relaxation rate and $q = 4_{\pi}n\sin(\theta/2)/\lambda$ is the scattering vector with λ the laser wavelength, n the refractive index of the solvent and θ the scattering angle.

The apparent hydrodynamic radius (R_{Happ}) of the nanoparticles was calculated from the Stokes–Einstein relation:

$$R_{Happ} = \frac{k_B\mathrm{T}}{6\pi\eta D_{app}} \qquad (2)$$

where k_B is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the solvent and D_{app} is the apparent diffusion coefficient of the nanoparticles.

Small-Angle X-ray Scattering.

SAXS experiments were performed at the beamline BM29 at ESRF (Grenoble, France) using a pixel detector (1M PILATUS). The SAXS setup utilizes a pinhole camera with a beam stop placed in front of a two-dimensional Frelon CCD detector. The X-ray scattering patterns were recorded for sample-to-detector distance of 2.9 m, using a monochromatic incident X- ray beam with an energy of E = 12.5 keV (λ = 0.1 nm). The available scattering vector range was q = 0.025−5 nm^{-1} (q =4π sin θ/λ, where 2θ is the scattering angle). Online corrections were applied for the detector, and the sample-to-detector distance, center, transmission, and incident intensity were calibrated. The isotropic scattering was azimuthally regrouped to determine the dependence of the scattered intensity I(q) on the scattering vector q in absolute units. The scattering from a capillary filled with Milli-Q water was measured as a background and

subtracted from the scattering signals of the samples. Prior to the experiment, a representative sample was checked to ensure lack of radiation damage.

Synthesis of propargyl-poly(2-ethyl-2-oxazoline)s

The polymerization mixture was prepared in accordance with literature.[1] 10 mL microwave vials were dried in a high temperature oven (180°C) for at least 2 hours, after which they were allowed to cool under vacuum, in the vacuum chamber of a glovebox. Inside the glovebox three mixtures were prepared, with a monomer:initiator (2-ethyl-2-oxazoline:propargyl benzenesulfonate) ratio of 20, 50 and 100. An appropriate amount of dry acetonitrile was added, to obtain a 4 M monomer solution. Subsequently, the vials were capped and polymerized in a microwave synthesizer at 140°C until a conversion of roughly 100% was reached (3.2 min for DP20, 8 min for DP50 and 16 minutes for DP100). The reaction was terminated with a methanolic solution of tetramethylammonium hydroxide. Afterwards, the polymers were isolated by precipitation in cold diethyl ether from dichloromethane. This precipitation cycle was repeated three times, after which the solvent traces were removed by placing the obtained solid in a vacuum oven at 50°C. ^{1}H NMR(300 MHz, $CDCl_3$): δ 4.4-4.15 (2H,m , C-CH_2-N), 3.65-3.00 (80H, m, N-CH_2-CH_2-N),2.50-2.14 (40H, m, OC-CH_2-CH_3), 1.18-0.89 (60H, m, CH_3); SEC: $PEtOx_{20}$: Mn:5200 Da Đ: 1.07, $PEtOx_{50}$: Mn=11700 Đ= 1.19 $PEtOx_{100}$: Mn=19700 Đ= 1.16; MALDI-TOF-MS: $PEtOx_{20}$: 2061.84Da = $[M+Na]^+$ $PEtOx_{50}$: 4441.9 Da = = $[M+Na]^+$ $PEtOx_{83}$: 8310.9 Da = $[M+Na]^+$

Synthesis of propargyl-poly(2-methyl-2-oxazoline)s

The polymerization mixture was prepared in accordance with literature.[1]10 mL microwave vials were dried in a high temperature oven (180°C) for at least 2 hours, after which they were allowed to cool under vacuum, in the vacuum chamber of a glovebox. Inside the glovebox two mixtures were prepared, with a monomer:initiator (2-methyl-2-oxazoline:propargyl benzenesulfonate) ratio of 100. An appropriate amount of dry acetonitrile was added, to obtain a 4 M monomer solution. Subsequently, the vials were capped and polymerized in a microwave synthesizer at 140°C until a conversion of roughly 100% was reached (10 minutes for DP100). The reaction was terminated with a methanolic solution of tetramethylammonium hydroxide. Afterwards, the polymers were isolated by precipitation in cold diethyl ether from dichloromethane. This precipitation cycle was repeated three times, after which the solvent traces were removed by placing the obtained solid in a vacuum oven at 50°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.41 (s, 2H, C-CH_2-N), 3.47 (m, 400H, N-CH_2-CH_2-N), 2.68 – 1.52 (m, 300H, OC-CH_3). SEC: Mn=18300 Đ= 1.29

Synthesis of methyl-poly(2-ethyl-2-oxazoline)s

The polymerization mixture was prepared in accordance with literature.[1] 10 mL microwave vials were dried in a high temperature oven (180°C) for at least 2 hours, after which they were allowed to cool under vacuum, in the vacuum chamber of a glovebox. Inside the glovebox two mixtures were prepared, with a monomer:initiator (2-ethyl-2-oxazoline:Methyl-*p*-toluenesulfonate) ratio of 100. An appropriate amount of dry acetonitrile was added, to obtain a 4 M monomer solution. Subsequently, the vials were capped and polymerized in a microwave synthesizer at 140°C until a conversion of roughly 100% was reached (16 minutes for DP100). The reaction was terminated with a methanolic solution of tetramethylammonium hydroxide. Afterwards, the polymers were isolated by precipitation in cold diethyl ether from dichloromethane. This precipitation cycle was repeated three times, after which the solvent traces were removed by placing the obtained solid in a vacuum oven at 50°C. NMR(300 MHz,

$CDCl_3$): δ, 3.65-3.00 (400H, m, N-C*H*$_2$-C*H*$_2$-N),2.50-2.14 (200H, m, OC-C*H*$_2$-CH_3), 1.18-0.89 (300H, m, C*H*$_3$); SEC: Mn=21500 Đ= 1.12

Complexation of polymers with C_{60} via HSVM

The complexation of the polymers with C60 was carried out in a Fritsch Mini-Mill Pulverisette 23 in a 10 mL stainless steel grinding bowl equipped with one 15 mm diameter grinding ball. The solid reagents were added and the mixture was agitated for 10 min at 50 Hz. Next 1 mL of milliQ water was added and the mixture was agitated again for 2 min at 50 Hz, in order to solubilize the solids. The resulting solution was then filtered over a 0.2µm PTFE pore filter providing the C_{60} nanoparticle solution. Next, this solution was freezedried, in order to isolate the formulation, allowing practical weighing for UV-vis and DLS characterization. The following molar equivalents of C_{60} to polymer were used 0.5:1; 1:1; 2:1. This procedure is also illustrated in the movie that is included in the supporting information.

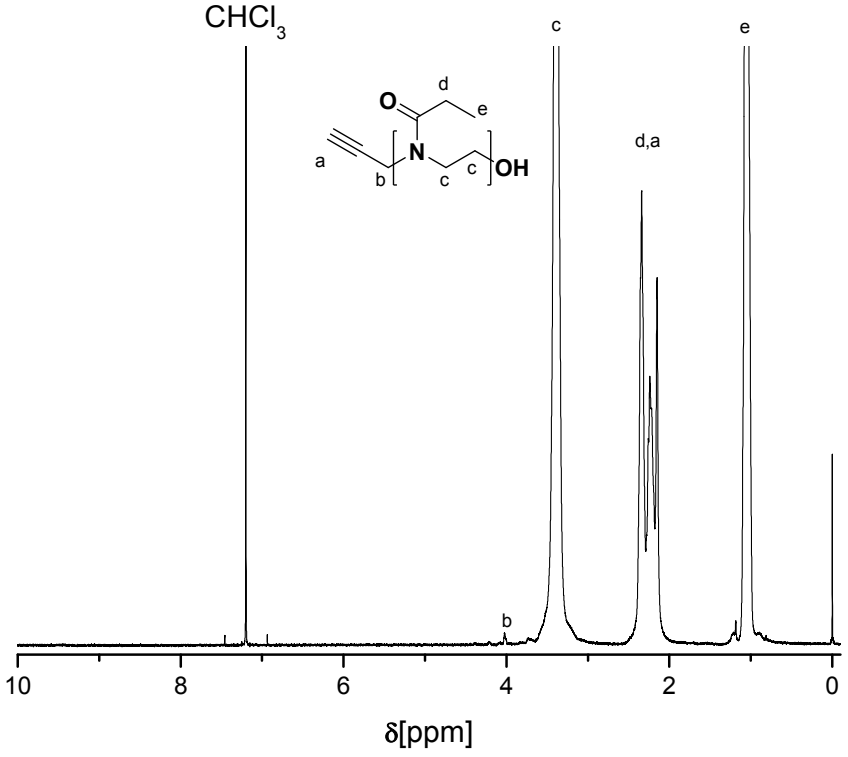

Figure S1: ^{1}H NMR spectrum of propargyl-poly(2-ethyl-2-oxazoline)-OH.

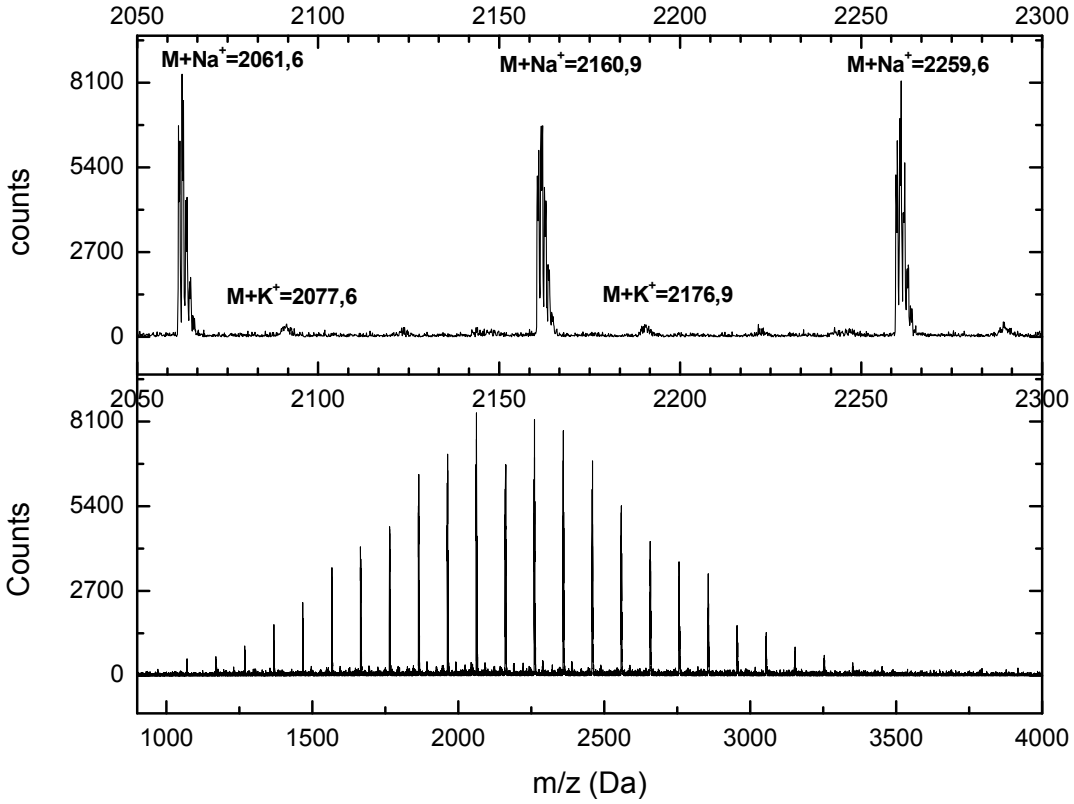

Figure S2: MALDI-TOF-MS spectrum of propargyl-PEtOx$_{20}$-OH.

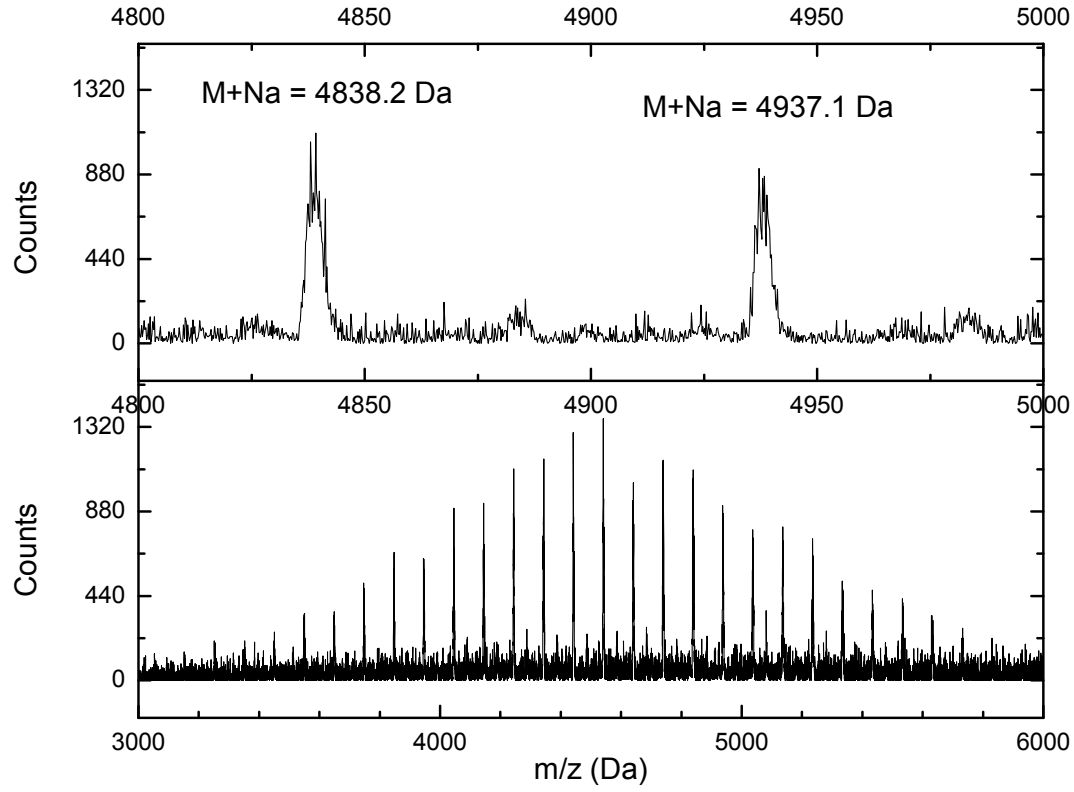

Figure S3: MALDI-TOF-MS spectrum of propargyl-PEtOx$_{50}$-OH.

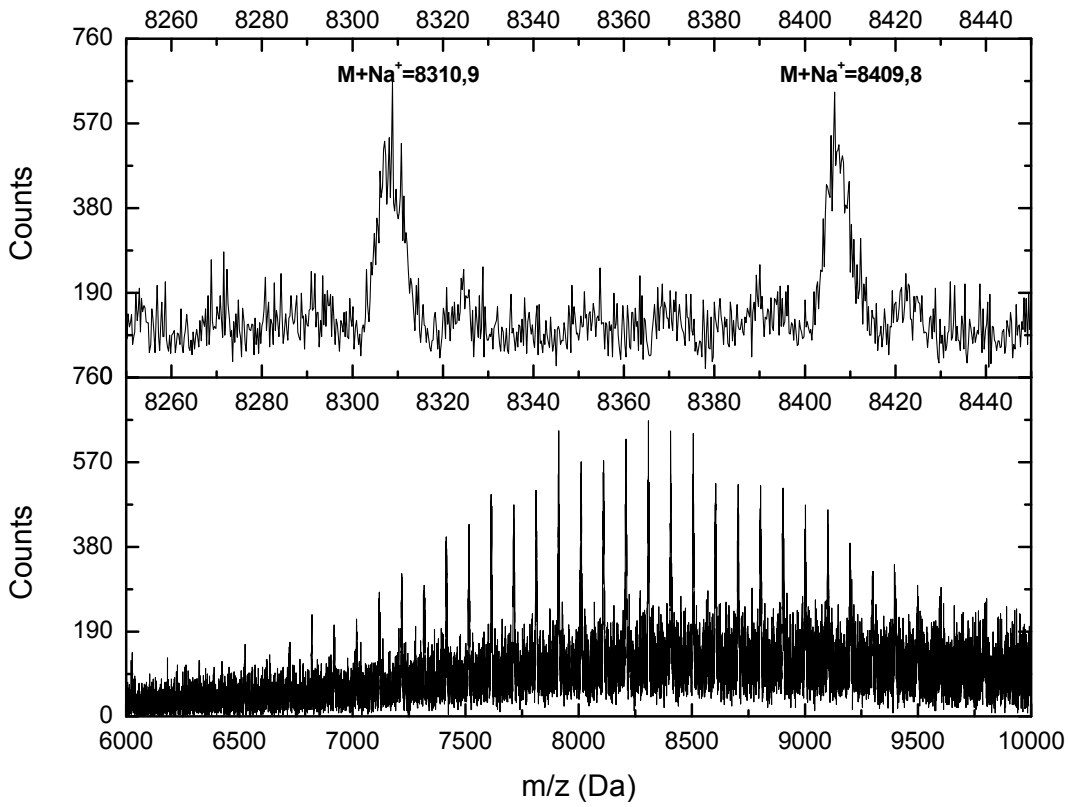

Figure S4: MALDI-TOF-MS spectrum of propargyl-PEtOx$_{100}$-OH.

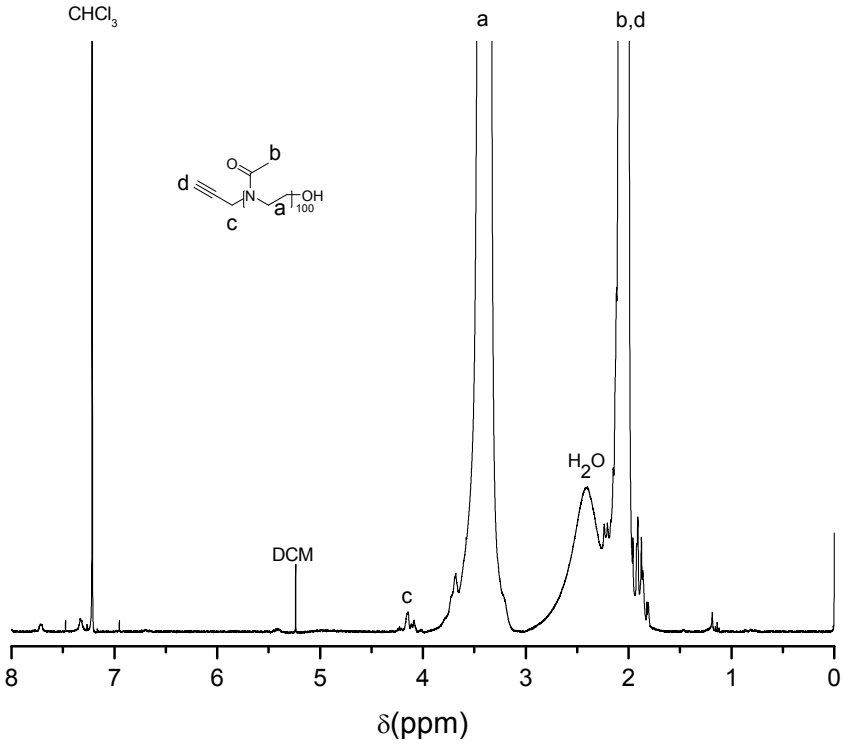

Figure S5: ^{1}H NMR spectrum of propargyl-PMeOx$_{100}$-OH.

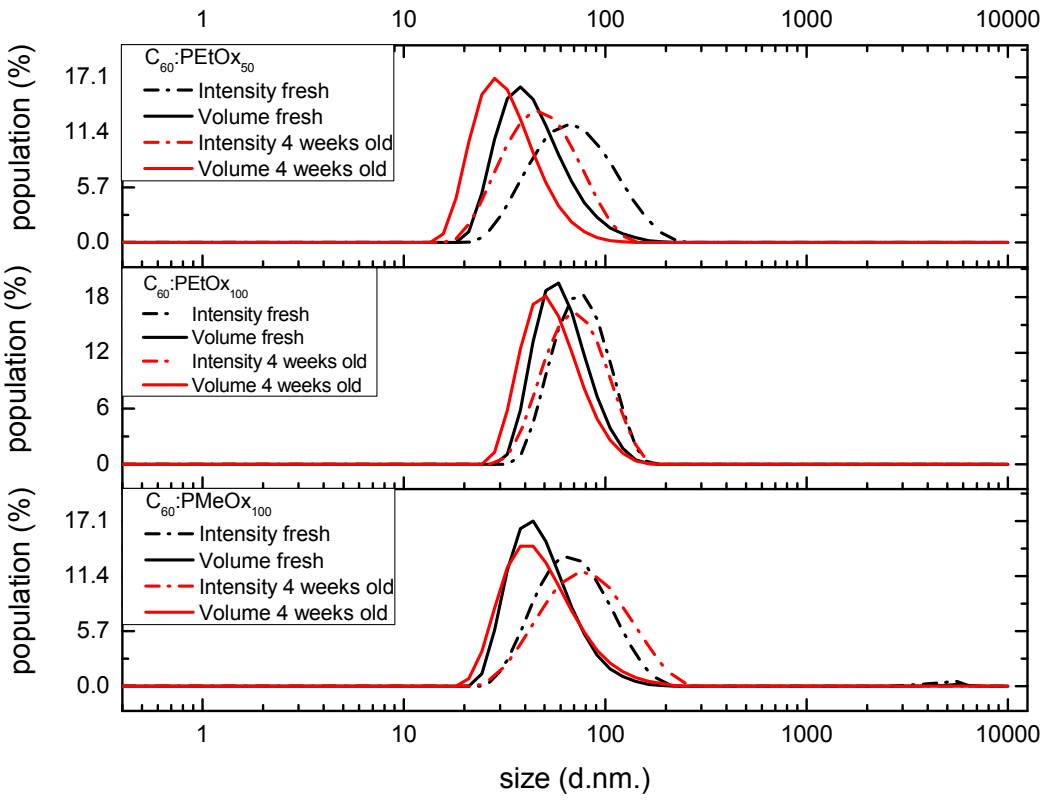

Figure S6: DLS measurements for C60:PAOx molar ratio 1:1.

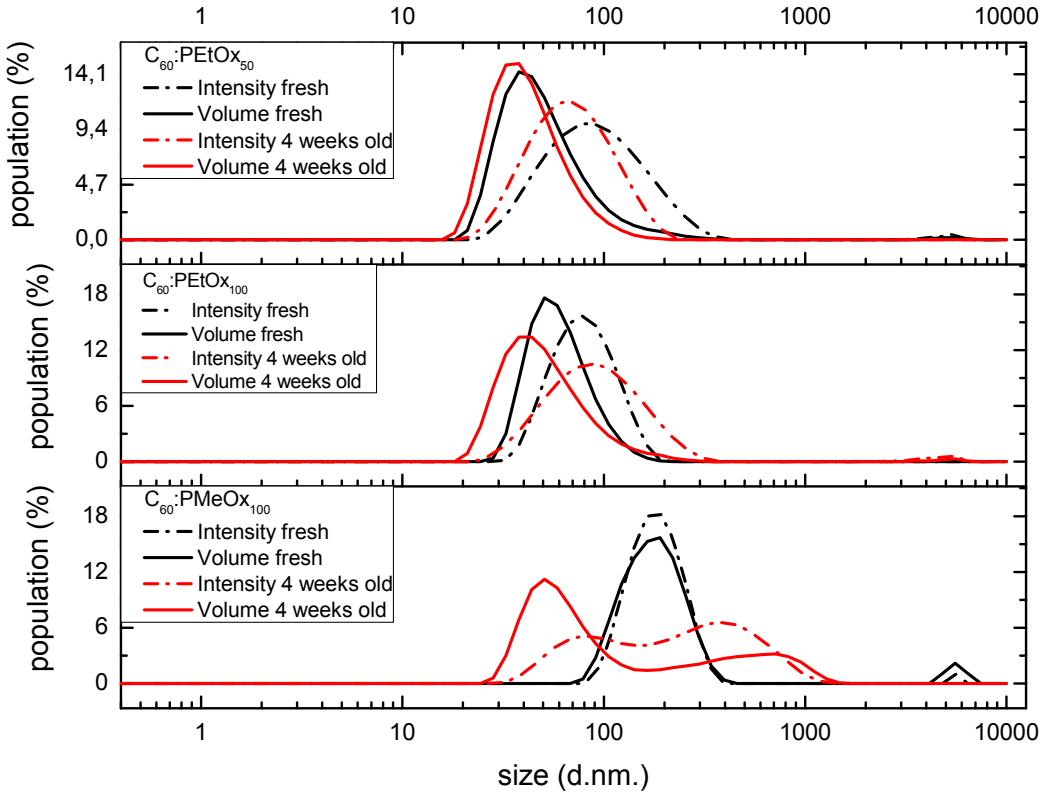

Figure S7: DLS measurements for C60:PAOx molar ratio 2:1.

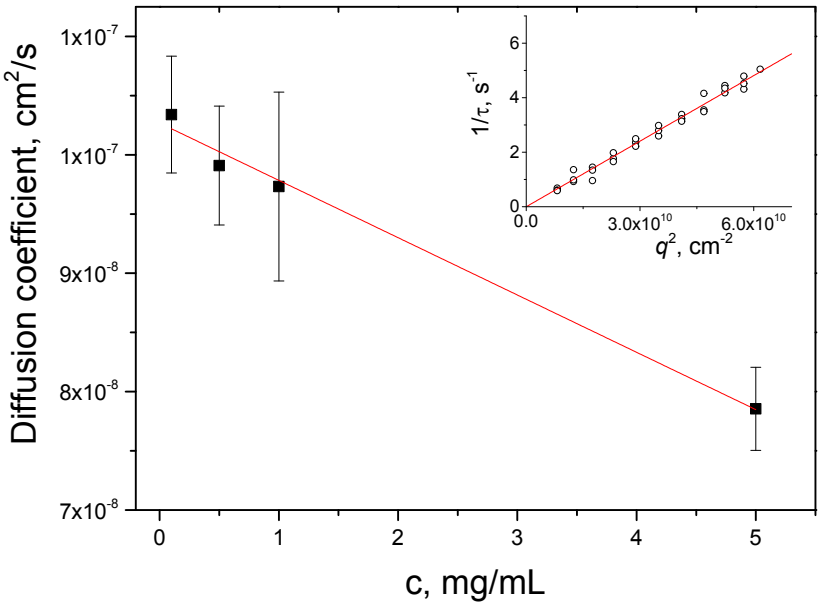

Figure S8: Concentration dependence of apparent diffusion coefficient for 0.5:1 C60:PEtOx DP100 molar ratio.

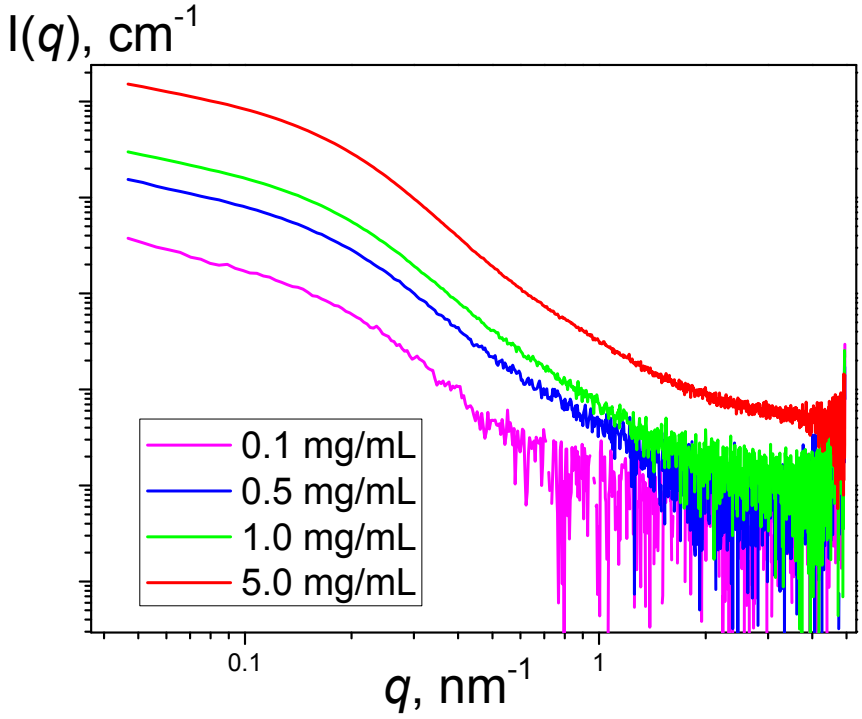

Figure S9: SAXS data for different concentrations for molar C_{60}:alkyne-PEtOx DP100 molar ratio 0.5:1 .

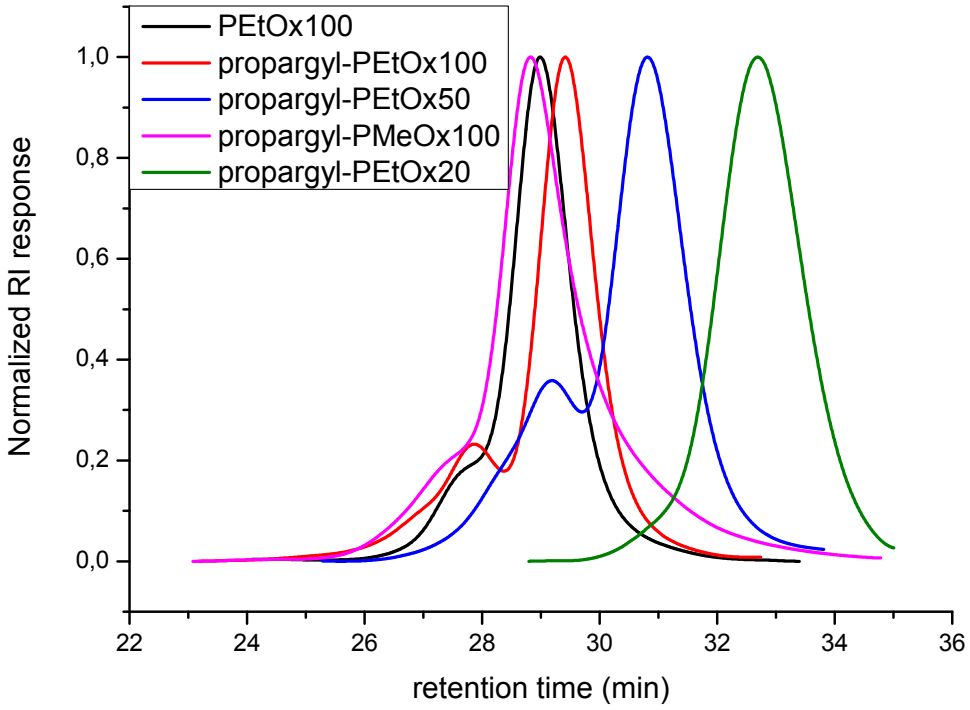

Figure S10: SEC overlay for the different polymers. The double molar mass shoulders at lower retention times can be attributed to chain transfer followed by chain coupling as commonly observed for the polymerization of 2-oxazoline monomers at 140°C.

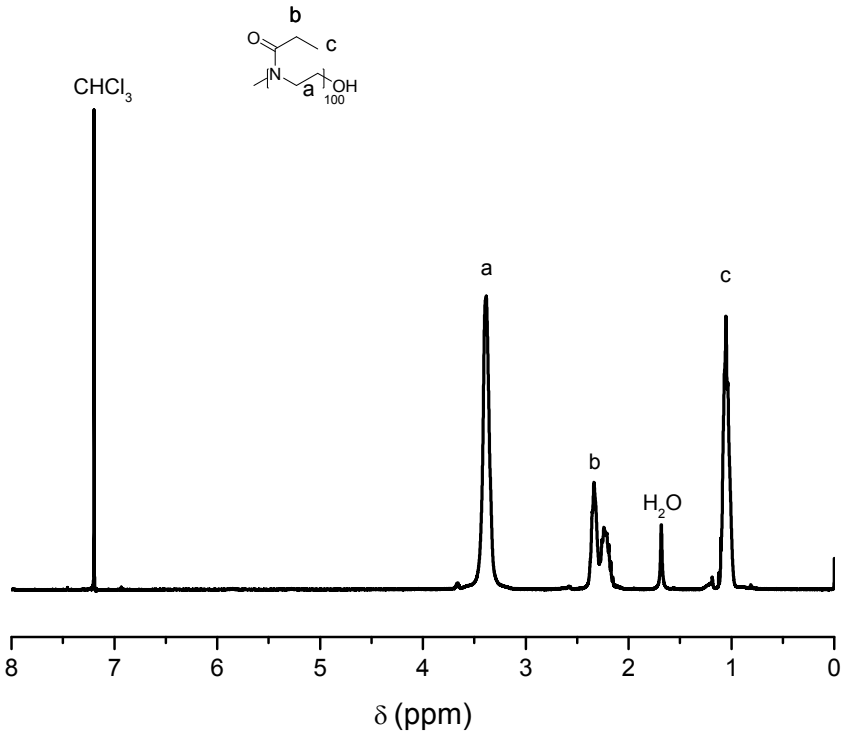

Figure S11: ^{1}H NMR spectrum of Me-PEtOx$_{100}$-OH.

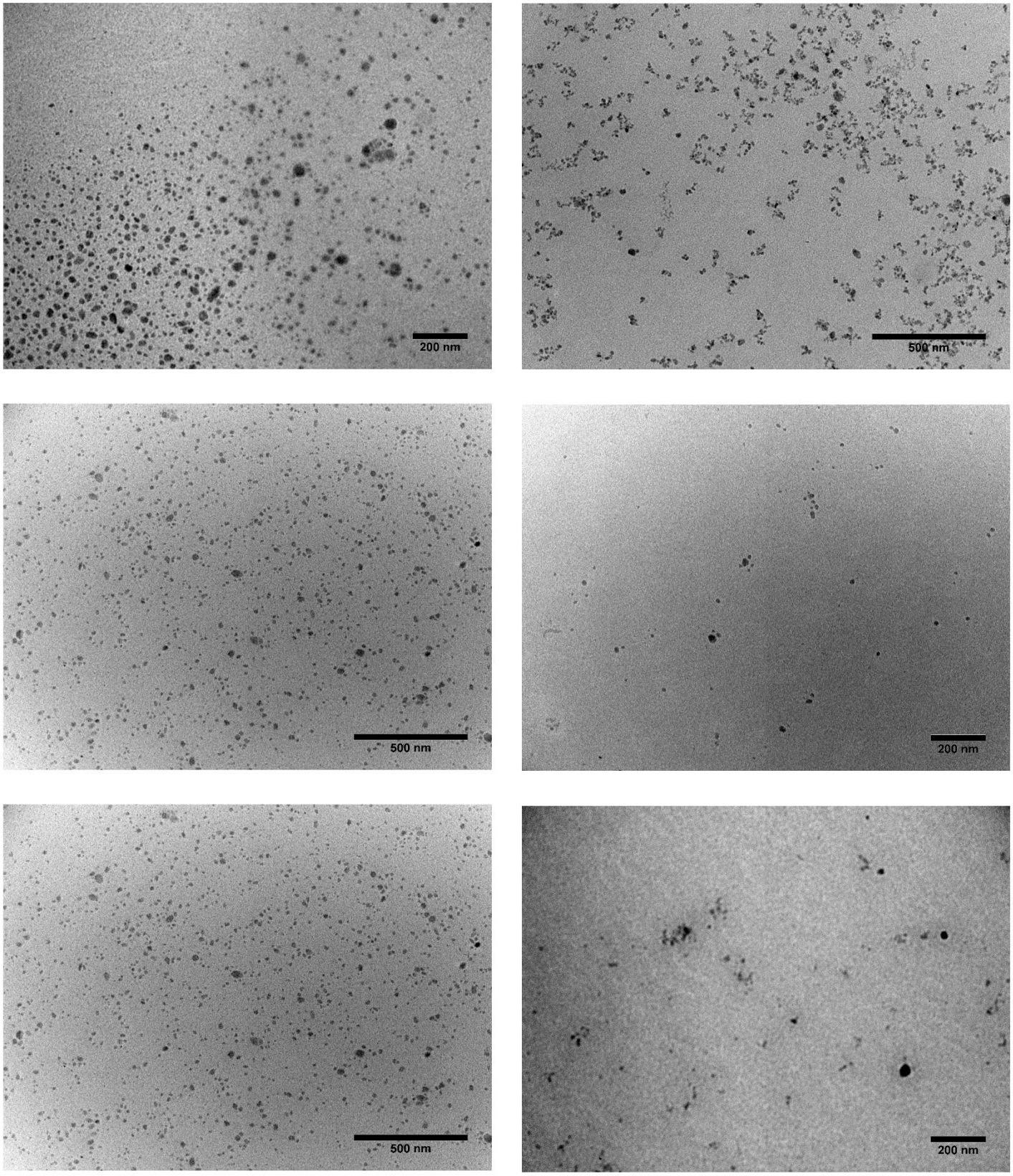

Figure S12: TEM images of C60 NPs, left column PEtOx DP 50 and right column PMeOX DP100, for 0.5:1 (top), 1:1 (middle) and 2:1 (bottom) molar ratio of (C_{60}:PAOx).

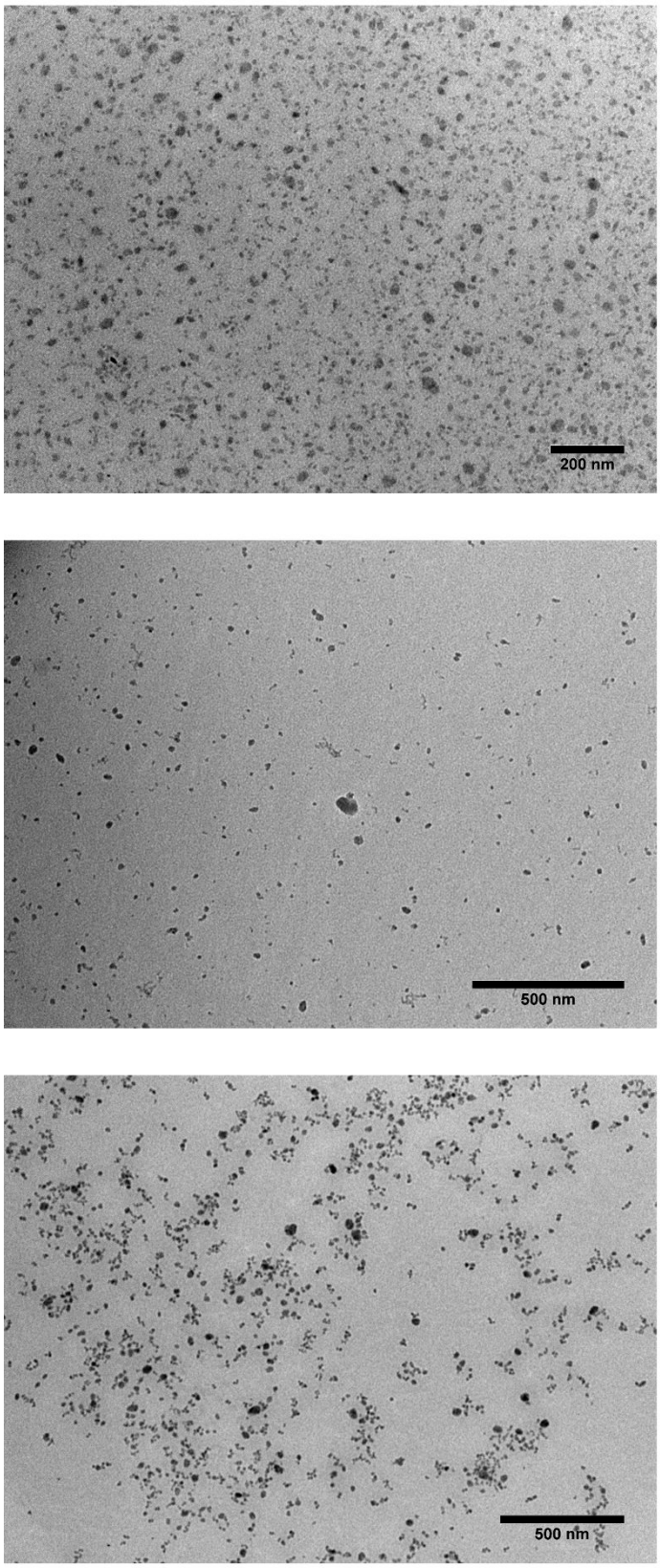

Figure S13: TEM images of C60 NPs with PEtOx DP 100 for 0.5:1 (top), 1:1 (middle) and 2:1 (bottom) molar ratio of (C_{60}:PAOx).

Table S1 Derived count rate values (kcps) values obtained from DLS of the different C_{60} PAOx formulations at different ratios prepared freshly and after 4 weeks.

Molar ratio (C_{60}:PAOx)	Polymer		
	kcps fresh (kcps 4 weeks)		
	PEtOx DP 50	PEtOx DP 100	PMeOx DP 100
0.5:1	2854 (2533)	8570 (11619)	4503 (5155)
1:1	11472(9559)	23654 (43353)	126763 (14058)
2:1	4960 (3248)	5471 (5688)	3445 (1485)

References:

1 Chapman,R.; Bouten, P. J. M.; Hoogenboom, R.; Jolliffe, K. A.; Perrier, S. Thermoresponsive Cyclic Peptide – Poly(2-ethyl-2-oxazoline) Conjugate Nanotubes. *Chem. Commun.*, 2013, **49**, 6522–6524.